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Short Communication

The Effects of Feed Withdrawal, Transport and Lairage on Intestinal Microflora in Broiler Chickens

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Abstract | This study investigated the effects of stressful pre-slaughter practices, namely feed withdrawal, road transportation, and lairage, on the caecal population *Salmonella* spp., *Campylobacter* spp., *E. coli*, *Clostridium* spp., and *Lactobacillus* spp. in broiler chickens. Thirty-five-day-old broiler chickens were subjected to either 0 h or 8 h of feed withdrawal, followed by 2 h or 4 h of road transportation. For each feed withdrawal-transportation subgroup, the birds were lairaged for 0 h or 3 h. *Salmonella* spp. and *Campylobacter* spp. were only detected in two and three birds, respectively. Because of the insufficient data, both types of bacteria were excluded from the study. Eight hours of feed withdrawal increased the population of *Lactobacilli* significantly but had a negligible effect on *E. coli* and *Clostridium* spp. counts. Prolonged duration of road transportation and lairage increased caecal counts of *E coli*. Birds road transported for 8 h had a higher caecal population of *Clostridium* spp. but lairage duration had no significance on the bacteria. In conclusion, the present findings strengthen the notion that minimising the stress associated with pre-slaughter practices is important to maintain the safety of broiler chicken meat.

Keywords | Feed withdrawal, Transport, Lairage, Caecal microflora, Broiler chickens

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INTRODUCTION

Before processing, broiler chickens are exposed to numerous potential stressors, including feed withdrawal, catching, crating, transportation, and lairaging. Work by Zhang et al. (2016), showed that stressful experiences disrupted the balance of intestinal microbiota and led to excessive growth of pathogens while decreasing the proportion of beneficial bacteria such as *Lactobacilli* and *Bifidobacteria*. Environmental stresses may increase intestinal permeability and pro-inflammatory activities, including other effects that aid in translocating bacteria from the intestinal lumen into the blood circulation. An increasing number of birds shedding *Salmonella* during transport that lasted for a maximum of 4 hours has been demonstrated (Mc Crea et al., 2006). Foodborne pathogens can be transmitted from one bird to another via faeces when the time between crating and holding before slaughter was prolonged (Larsen et al., 2014). Arsenault et al. (2007) indicated that pathogens ingested before, during, and after

crating and transportation may colonise the ceca, where they may be stored throughout the processing. Furthermore, feed withdrawal before harvesting may change the populations of cecal aerobic bacteria, *Enterobacteriaceae*, and lactic acid bacteria (Hinton et al., 2000). Burkholder et al. (2008) indicated that stress may cause enteric pathogen colonisation in food animals, enable horizontal pathogen transmission across animals, increase pathogen shedding, and contribute to carcass contamination during processing.

Earlier work on the effects of pre-slaughter management on caecal microflora in broilers was mainly conducted under temperate conditions (Jacobs-Reitsma et al., 1995; Pezzotti et al., 2003). Presently, there is no documented work on the influence of pre-slaughter stresses on gut microflora in broilers under a hot and humid tropical environment. The intestinal tract of poultry is exceptionally responsive to any stress (Yang et al., 2011; Cengiz et al., 2015; Bello et al., 2018). It is well-documented that heat stress may alter gut microbiota in poultry (Rostagno, 2020; He et al., 2021). Caffrey et al. (2017) suggested that heat load is the major factor that causes stress in broilers during transit. Hence, we hypothesised that the effects of pre-slaughter management on the gut microflora of broilers are more severe in the hot environment. Consequently, this experiment was performed to study the effects of different durations of feed withdrawal, transportation, and lairage on the caecal populations of *Salmonella* spp., *Campylobacter* spp., *E. coli*, *Clostridium* spp., and *Lactobacillus* spp. in broiler chickens under the hot and humid tropical environment.

MATERIAL AND METHODS

Animal, housing, and husbandry

A total of 240 day-old male broiler chicks (Cobb 500) were obtained from a commercial hatchery. The mean body weight of the chicks was 45 g. The chicks were raised on floor pens with wood shaving as litter material in a naturally ventilated house and fed commercial broiler feed.

Experimental design

The experimental design was completely randomised with a 2 (two durations of feed withdrawal) x 2 (two durations of road transportation) x 2 (two durations of lairage) factorial arrangement of treatments.

Transportation and sampling

On day 35, equal numbers of chickens were subjected to either 0 h (6 pens) (0FW) or 8 h (6 pens) (8FW) of feed withdrawal before catching (2300 h). Drinking water was available ad libitum. Following feed withdrawal, an equal number of birds from each feeding regimen (120 per feeding regimen) were removed from their cages and placed in plastic crates (0.80 × 0.60 × 0.31 m) at 10 birds to each crate. The crates were loaded onto an open truck, and an equal number of birds were either road transported for 2 h (an approximate distance of 160 km) (6 crates of 60 birds) (2RT) or 4 h (an approximate distance of 320 km) (6 crates of 60 birds) (4RT) with an average speed of 80 km/h. At the time of transportation, the ambient temperature and relative humidity were 26°C and 70% humidity, respectively. Following transportation, the crates were unloaded and placed in a naturally ventilated holding area with aluminium roofing for 0 (0L) or 3 h (3L). There were three crates of 10 birds for each feed withdrawal-transportation-lairage duration subgroup. After exsanguination, according to the halal method Farouk et al. (2014), the caecal contents were immediately frozen in liquid nitrogen and stored at –80°C. Bacterial quantification analysis was later conducted on these caecal contents.

Bacterial Quantification

DNA was extracted from caecal content samples using a QIAamp Fast DNA Stool Mini Kit (Qiagen Inc., Valencia, CA, USA) following the manufacturer's instructions. The purity and concentration of the extracted DNA samples were assessed spectrophotometrically using a Nano drop ND1000. The populations of *Lactobacillus*, *E. coli*, *Salmonella*, *Campylobacter* and *Clostridium* were determined by a real-time PCR (RT-PCR) assay with Maxima SYBER Green qPCR Master Mix (Thermo Fisher Scientific, Waltham, MA, USA) in CFX96 (Bio-Rad) real-time PCR System. The set of primers and the respective PCR conditions were previously described in the quoted reference (Rezaei et al., 2015). The number of bacterial DNA copies was obtained by interpolating the standard curve's threshold cycle value (*Ct*).

Statistical Analysis

The experimental data were analysed as a 2 x 2 x 2 factorial design using the GLM procedure of SAS software (SAS Institute Inc. Cary, NC, USA). The statistical model included the effects of duration of feed withdrawal (0FD and 8FD), transit (2RT and 4RT) and lairage (0L and 3L) and their interactions. When interactions between main effects were significant, comparisons were made within each experimental variable. Statistical significance was assumed at $p<0.05$.

RESULTS

Salmonella spp. and *Campylobacter* spp. were only detected in two and three birds, respectively. Because of the insufficient data, both types of bacteria were excluded from the study. The effects of feed withdrawal, transportation, and lairage durations on the populations of *E. coli*, *Clostridium* spp., and *Lactobacillus* spp. are presented in Table 1. Feed withdrawal for 8 h significantly increased caecal *E. coli*

Table 1: Effects of feed withdrawal, transport, lairage, and their interactions on cecal populations of *Lactobacillus* spp., *E. coli* and *Clostridium* spp. in broilers at 35 days

Transportation						
2 h	25.53[b]	0.38	13.80[b]	0.29	23.60[a]	0.57
4 h	26.80[a]	0.36	14.78[a]	0.25	22.73[a]	0.56
Lairage						
0 h	25.71[b]	0.40	13.97[a]	0.25	22.13[b]	0.52
3 h	26.69[a]	0.35	14.48[a]	0.30	24.20[a]	0.58
Analysis of Variance (*P*-value)						
Feed Withdrawal	0.1431		0.6247		0.0498	
Transport	0.0109		0.0102		0.2461	
Lairage	0.0527		0.1511		0.0067	
Feed Withdrawal x Transport	0.0614		0.6424		0.0742	
Feed Withdrawal x Lairage	0.789		0.1758		0.8959	
Transport x Lairage	0.5806		0.9294		0.0287	

a,b Means within a column with no common superscripts are significantly different at $P < 0.05$.

Table 2: Cecal *Lactobacillus* spp. population (log10 cfu/g) when lairage x transportation interactions were significant in broilers at 35 days of age

	Transportation		**SEM[2]**	***P*-value**
Treatment	2h	4h		
Lairage				
0 h	21.74[aB]	22.52[aA]	0.70	0.4541
3 h	25.47[aA]	22.94[bA]	0.46	0.0274
SEM[3]	0.72	0.84		
P-Value	0.0006	0.7192		

a,b Means within a row with no common superscripts are significantly different at P < 0.05

A,B Means within a column with no common superscripts are significantly different at P < 0.05

2SEM = Standard error of the mean for lairage × transportation effect (n=12)

3SEM = Standard error of the mean for transportation × lairage effect (n=12)

population compared to their 0FW counterparts. The 2RT broilers showed significantly lower caecal *E. coli* counts than the 4 RT group. Lairage duration had a negligible effect on caecal *E. coli* population. Broilers subjected to 4RT had significantly higher counts of caecal *Clostridium* spp. than those of 2RT. Feed withdrawal and lairage durations did not affect caecal *Clostridium* spp. counts. In Table 2, significant lairage duration x transportation duration interactions were noted for caecal *Lactobacillus* spp. population. Lairaging birds for 3 h significantly (P<0.05) reduced cecal *Lactobacillus* spp. population in 4RT birds compared to their 2RT counterparts. However, both 2RT and 4RT broilers that were not lairaged had similar caecal *Lactobacillus* spp. counts.

DISCUSSION

The low number of chickens that tested positive for *Salmonella* spp. and *Campylobacter* spp. in the present study could be attributed to the reliable source of day-old chicks and the hygienic condition of our animal laboratory. Feed withdrawal before harvesting could lead to a decrease in nutrient concentration for microbes inhabiting the digestive tract and eventually alter the composition of the intestinal microflora in birds (Hashemi et al., 2012). Shafiei et al. (2018) subjected broilers to 6 h, 8 h, 10 h and 12 h of feed deprivation and noted negligible effect on the ileal *E. coli* population. However, the authors reported that 12 h of fasting increased intestinal *Lactobacillus* spp. counts. The present findings suggested that 8FD increased caecal counts of *Lactobacillus* spp. counts but no influence on caecal *E. coli* and *Clostridium spp*. populations. Changes in the native microflora of the ceca during feed withdrawal may be associated with a decrease in the volume of digested material entering the colon (Birger, 2014) and to changes in native bacterial flora of the contents arriving in the colon from the upper alimentary tract (Rinttila et al., 2013).

It is well documented that the magnitude of physiological stress in broilers increased with journey and lairage length (Schwartzkopf-Genswein, 2012). In general, our results show that longer transit and lairage time increased the caecal counts of *E. coli* and decreased the intestinal population of *Lactobacillus*. While lairage duration had a negligible influence on the cecal *Clostridium* population, the 4RT birds showed higher counts of the bacteria than their 2RT counterparts. Beneficial bacteria work as a barrier to pathogen colonisation, and an alteration in their population may increase the susceptibility of birds to colonisation by enteric pathogens (Michael et al., 2019). Such alterations in the protective barrier can also be related to the perturbation of the intestinal mucus lining and morphology following stressful conditions (Burkholder et al., 2008).

CONCLUSION

The present findings show that prolonged transportation and lairage duration increased the caecal populations of foodborne pathogens and reduced the counts of beneficial bacteria. Hence, extra careful attention must be paid to the feed withdrawal, catching, crating, transportation, and lairage procedures of broiler chickens to alleviate unnecessary stress and reduce the gut pathogens' population

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CONFLICT OF INTEREST

The authors declare no conflict of interests.

AUTHORS CONTRIBUTION

All authors contributed equally.

REFERENCES

Arsenault J., Letellier A., Quessy S., Boulianne M. (2007). Prevalence and risk factors for *Salmonella* and *Campylobacter* spp. carcass contamination in broiler chickens slaughtered in Quebec, Canada. J. Food Prot., 70(8): 1820–1828. https://doi.org/10.4315/0362-028x-70.8.1820.

Bello A. U., Zulkifli I., Goh Y. M., Awad E. A., Soleimani A. F. (2018). Gut microbiota and transportation stress response affected by tryptophan supplementation in broiler chickens. Ital. J. Anim. Sci. 17: 107–13. https://doi.org/10.1080/1828051X.2017.1340814.

Birger S. (2014). Function of the digestive system. J. Appl. Poult. Res., 23(2): 306–314. https://doi.org/10.3382/japr.2014-00937.

Burkholder K., Thompson K., Einstein M., Applegate T., Patterson J. (2008). Influence of stressors on normal intestinal microbiota, intestinal morphology, and susceptibility to *Salmonella enteritidis* colonisation in broilers. Poult. Sci. 87: 1734-1741. https://doi.org/10.3382/ps.2008-00107

Caffrey N., Dohoo I., Cockram M. (2017). Factors affecting mortality risk during transportation of broiler chickens for slaughter in Atlantic Canada. Prev. Vet. Med., 147, 199–208. https://doi.org/10.1016/j.prevetmed.2017.09.011.

Cengiz O. K., Oksal B.H., Tatlı O., Sevim O., Ahsan U., Uner A. G., Uluta P. A., Beyaz D., Buyukyoruk S., Yakan A., Onol A. G. (2015). Effect of dietary probiotic and high stocking density on the performance, carcass yield, gut microflora, and stress indicators of broilers. Poult. Sci. 94: 2395-2403. https://doi.org/10.3382/ps/pev194.

Farouk M., Al-Mazeedi H., Sabow A., Bekhit A., Adeyemi K., Sazili A., Ghani A. (2014). Halal and kosher slaughter methods and meat quality: A review. Meat Sci., 98(3): 505–519. https://doi.org/10.1016/j.meatsci.2014.05.021.

Hashemi S., Zulkifli I., Davoodi H., Zunita Z., Ebrahimi M. (2012). Growth performance, intestinal microflora, plasma fatty acid profile in broiler chickens fed herbal plant (Euphorbia hirta) and mix of acidifiers. Anim. Feed Sci. Technol., 178(3–4): 167–174. https://doi.org/10.1016/J.anifeedsci.2012.09.006.

He Y., Maltecca C., Tiezzi F. (2021). Potential Use of Gut Microbiota Composition as a Biomarker of Heat Stress in Monogastric Species: A Review. Animals., 11(6): 1833. https://doi.org/10.3390/ani11061833.

Hinton A., Buhr R., Ingram K. (2000). Reduction of *Salmonella* in the crop of broiler chickens subjected to feed withdrawal. Poult. Sci., 79(11): 1566–1570. https://doi.org/10.1093/ps/79.11.1566.

Jacobs-Reitsma W., Van de Giessen A., Bolder N., Mulder R. (1995). Epidemiology of Campylobacter spp. at two Dutch broiler farms. Epidemiol. Infect., 114(3): 413-421. https://doi.org/10.1017/S0950268800052122.

Larsen M. H., Dalmasso M., Ingmer H., Langsrud S., Malakauskas M. *et al*., (2014). Persistence of foodborne pathogens and their control in primary and secondary food production chains. Food Control., 44: 92–109. https://doi.org/10.1016/j.foodcont.2014.03.039.

McCrea B. A., Tonooka K. H., VanWorth C., Atwill E., Schrader J., Boggs C. (2006a). Prevalence of *Campylobacter* and *Salmonella* species on farm, after transport, and at processing in specialty market poultry. Poult. Sci., 85(1): 136–143. https://doi.org/10.1093/ps/85.1.136.

Michael H. K. (2019). The effect of microbiome modulation on the intestinal health of poultry. Anim. Feed Sci. Technol., 250: 32–40. https://doi.org/10.1016/j.anifeedsci.2018.10.008.

Pezzotti G., Serafin A., Luzzi I., Mioni R., Milan M., Perin R. (2003). Occurrence and resistance to antibiotics of Campylobacter jejuni and Campylobacter coli in animals and meat in northeastern Italy. Int. J. Food Microbiol., 82(3): 281-287. https://doi.org/10.1016/S0168-1605(02)00314-8.

Rezaei S., Jahromi M. F., Liang J. B., Zulkifli I., Farjam A. S., Laudadio V., Tufarelli V. (2015). Effect of oligosaccharides extract from palm kernel expeller on growth performance, gut microbiota and immune response in broiler chickens. Poult. Sci., 94(10): 2414-2420. http://doi.org/10.3382/ps/pev216.

Rinttila T., Apajalahti J. (2013). Intestinal microbiota and metabolites: Implications for broiler chicken health and performance. J. Appl. Poult. Res., 22(3): 647–658. https://

doi.org/10.3382/japr.2013-00742.

Rostagno M. H. (2020). Effects of heat stress on the gut health of poultry. J. Anim. Sci. 98(4): skaa090, https://doi.org/10.1093/jas/skaa090.

Schwartzkopf-Genswein K., Faucitano L., Dadgar S., Shand P., Gonzalez L. Crowe T. (2012). Road transport of cattle, swine and poultry in North America and its impact on animal welfare, carcass and meat quality: A review. Meat Sci., 92(3): 227–243. https://doi.org/10.1016/j.meatsci.2012.04.010.

Shafiei A., Khavarinezhad S., Javandel F., Nosrati M., Seidavi A., Diarra S. S. (2018). Effects of duration of early feed withdrawal and re-feeding on growth, carcass traits, plasma constituents and intestinal microflora of broiler chickens. J. Appl. Anim. Res., 46(1), 1358–1362. https://doi.org/10.1080/09712119.2018.1509004.

Yang X. J., Li W. L., Feng Y., Yao J. H. (2011). Effects of immune stress on growth performance, immunity, and cecal microflora in chickens. Poult. Sci. 90: 2740-2746. https://doi.org/10.3382/ps.2011-01591.

Zhang L., Zhang L., Zhan X. (2016). Effects of dietary supplementation of probiotic, *Clostridium butyricum* on growth performance, immune response, intestinal barrier function, and digestive enzyme activity in broiler chickens challenged with *Escherichia coli* K88, J. Anim. Sci. Biotech. 7(1): 1-9. https://doi.org/10.1186/s40104-016-0061-4.